

## c-MER, Active

Recombinant mouse protein expressed in Sf9 cells

### Catalog # M51-172G

Lot # B2107-8

### Product Description

Recombinant mouse c-MER (528-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [NM\\_008587](#).

### Gene Aliases

MERTK, Eyk; Mer; nmf12; Nyk

### Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, 25% glycerol.

### Storage and Stability

Store product at  $-70^{\circ}\text{C}$ . For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

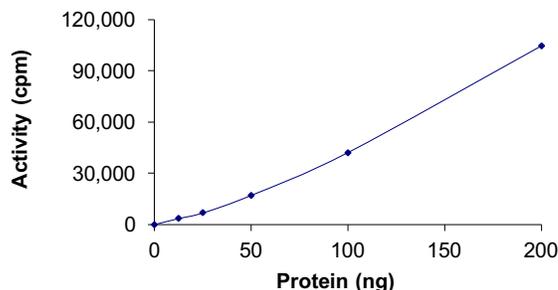
### Scientific Background

c-MER is a tyrosine kinase and a member of the MER/AXL/TYRO3 receptor kinase family (1). The c-MER gene encodes a transmembrane protein with two fibronectin type-III domains, two Ig-like C2-type domains, and one tyrosine kinase domain. Functional knockout of c-MER in mice shows the mice to have macrophages deficient in the clearance of apoptotic thymocytes thereby demonstrating the critical role for c-MER in the engulfment and efficient clearance of apoptotic cells. Defects in c-MER have been associated with retinitis pigmentosa, a phenotype that can be reversed by replacement of the c-MER gene product (2).

### References

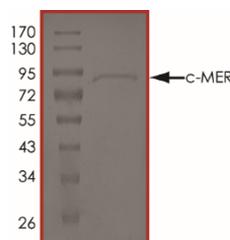
- Graham, D. K. et al: Cloning and mRNA expression analysis of a novel human protooncogene, c-mer. *Cell Growth Differ.* 5: 647-657, 1994.
- Gal, A. et al: Mutations in MERTK, the human orthologue of the RCS rat retinal dystrophy gene, cause retinitis pigmentosa. *Nature Genet.* 26: 270-271, 2000.

### Specific Activity



The specific activity of c-MER was determined to be **32 nmol/min/mg** as per activity assay protocol.

### Purity



The purity of c-MER protein was determined to be **>80%** by densitometry, approx. MW **83kDa**.

## c-MER, Active

Recombinant mouse protein expressed in Sf9 cells

Catalog Number	M51-172G
Specific Activity	32 nmol/min/mg
Lot #	B2107-8
Purity	>80%
Concentration	0.1 µg/µl
Stability	1yr at $-70^{\circ}\text{C}$ from date of shipment
Storage & Shipping	Store product at $-70^{\circ}\text{C}$ . For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

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# Activity Assay Protocol

## Reaction Components

### Active Kinase (Catalog #: M51-172G)

Active mouse c-MER (0.1 $\mu$ g/ $\mu$ l) diluted with Kinase Dilution Buffer IV (Catalog #: K24-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active c-MER for optimal results).

### Kinase Dilution Buffer IV (Catalog #: K24-09)

Kinase Assay Buffer II (Catalog #: K02-09) diluted at a 1:4 ratio (5X dilution) with final 50ng/ $\mu$ l BSA solution.

### Kinase Assay Buffer II (Catalog #: K02-09)

Buffer components: 25mM MOPS, pH 7. 2, 12.5mM  $\beta$ -glycerol-phosphate, 20mM MgCl<sub>2</sub>, 12.5mM MnCl<sub>2</sub>, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

### [<sup>33</sup>P]-ATP Assay Cocktail

Prepare 250 $\mu$ M [<sup>33</sup>P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150 $\mu$ l of 10mM ATP Stock Solution (Catalog #: A50-09), 100 $\mu$ l [<sup>33</sup>P]-ATP (1mCi/100 $\mu$ l), 5.75ml of Kinase Assay Buffer II (Catalog #: K02-09). Store 1ml aliquots at -20°C.

### 10mM ATP Stock Solution (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer II (Catalog #: K02-09). Store 200 $\mu$ l aliquots at -20°C.

### Substrate (Catalog #: P61-58)

Poly (4:1 Glu, Tyr) synthetic peptide substrate diluted in distilled H<sub>2</sub>O to a final concentration of 1mg/ml.

## Assay Protocol

- Step 1.** Thaw [<sup>33</sup>P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2.** Thaw the Active mouse c-MER, Kinase Assay Buffer, Substrate and Kinase Dilution Buffer on ice.
- Step 3.** In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20 $\mu$ l:
  - Component 1.** 10 $\mu$ l of diluted Active mouse c-MER (Catalog #M51-172G)
  - Component 2.** 5 $\mu$ l of 1 mg/ml stock solution of substrate (Catalog #P61-58)
  - Component 3.** 5 $\mu$ l distilled H<sub>2</sub>O (4°C)
- Step 4.** Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H<sub>2</sub>O.
- Step 5.** Initiate the reaction by the addition of 5  $\mu$ l [<sup>33</sup>P]-ATP Assay Cocktail bringing the final volume up to 25 $\mu$ l and incubate the mixture in a water bath at 30°C for 15 minutes.
- Step 6.** After the 15 minute incubation period, terminate the reaction by spotting 20  $\mu$ l of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
- Step 7.** Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled H<sub>2</sub>O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.
- Step 8.** Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
- Step 9.** Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

### Calculation of [<sup>33</sup>P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5  $\mu$ l [<sup>33</sup>P]-ATP / pmoles of ATP (in 5  $\mu$ l of a 250  $\mu$ M ATP stock solution, i.e., 1250 pmoles)

### Kinase Specific Activity (SA) (pmol/min/ $\mu$ g or nmol/min/mg)

Corrected cpm from reaction / [(SA of <sup>33</sup>P-ATP in cpm/pmol)\*(Reaction time in min)\*(Enzyme amount in  $\mu$ g or mg)]\*[(Reaction Volume) / (Spot Volume)]

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